HEMORRHAGIC FEVER WITH RENAL SYNDROME
(KOREAN HEMORRHAGIC FEVER)

ANNUAL SUMMARY REPORT

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Hantavirus is ubiquitous in the world and total number of reported HFRS patient in Euro-Asia is about 200,000 with 5-7% mortality annually. Hemorrhagic fever with renal syndrome (HFRS) is an important military problem since large epidemics of HFRS occurred among soldiers in the many past wars and although predominantly associated with field mice in rural areas, it is now being recognized that urban rats and laboratory rats are also reservoirs of HFRS in many parts of the world.

Therefore, global survey of distribution of hantaviruses and surveillance of occurrence of HFRS are important for prevention of this highly fatal disease. It is also important to investigate antigenic differences of strains of Hantavirus isolated from rodents caught in non-endemic areas of the world because HFRS patient had never been documented in many areas despite our finding of positive rodents there.

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The methods for diagnosis of HFRS, isolation of hantaviruses from man and rodents, and intraspecific transmission of hantaviruses in rodents are described previously.

There were 701 cases of HFRS in Korea in 1987 and recently no. of HFRS patients are increasing in urban cities, and large epidemic of scrub typhus occurred about before and during the epidemic season of HFRS.

A new serotype of Hantavirus, Maaji virus, was isolated from Apodemus agrarius caught in a village in Kyunggido, west coast of S. Korea. Four strains of Hantaan virus were also isolated from Apodemus agrarius captured in Jinhae, Kyungsangnamdo, Korea where HFRS was not reported previously.

In a study of global survey of HFRS, Hantavirus infection was demonstrated among laboratory personnel, experimental rats and wild mice, C. musculinus in Argentina for the first time in South America.

Seoul virus was isolated from a naturally infected Syrian hamster from a local animal farm for the first time. Inbred hamsters were broad spectrum animal to support multiplication of various strains of hantaviruses and 2 out of 4 PD4 inbred hamsters died about 20 days after inoculation of a strain of Hantaan virus. The evidences show that some strain of inbred hamsters could be used as an animal model for HFRS.

SUMMARY

There were 701 cases of hospitalized (HFRS) patients serologically confirmed at our laboratory and, 163 and 5 of them were ROK Army and US Army soldiers, respectively in Korea, 1987. Epidemic of scrub typhus occurred about a month before the peak of HFRS and 209 scrub typhus patients were diagnosed at our laboratory among 1,530 suspect HFRS in 1987.

Serologic survey of infected wild *Apodemus agrarius* with Hantaan virus, *R. tsutsugamushi* and *Leptospira interogans* in the U.S. Marine and ROK Army camps in Wuncheon, Kyunggido where exercise "Bearhunt" has been held for several years showed higher infection rate of mice with the agents in the US Marine camp than ROK Army camp.

A new serotype of *Hantavirus* was identified and 4 strains of Hantaan virus were isolated from *Apodemus agrarius* caught in Jinhae, Kyungsangnamdo, Korea where HFRS were not reported previously.

As a part of global survey of HFRS, *Hantavirus* infection was demonstrated for the first time not only among laboratory personnel but also in experimental rats and wild mice, *C. musculinus* in Argentina.

A strain of Seoul virus was isolated from a Syrian hamster purchased from a local animal farm. In our limited study, inbred hamsters were broad spectrum animals to support multiplication of hantaviruses and 2 out of 4 inbred hamsters PD4 died about 20 days after inoculation of a strain of Hantaan virus.

FOREWORD

In conducting the research described in this report, the investigators (s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animals Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

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INTRODUCTION

During the Korean War more than 3,200 United Nations troops in Korea developed a rare hemorrhagic fever which attracted worldwide attention (1). Since then it has been known as Korean hemorrhagic fever (KHF). This disease is an important military problem because large epidemics occurred among soldiers during several wars. More than 12,600 cases of epidemic hemorrhagic fever (EHF) occurred among one million Japanese soldiers in Manchuria (2) and several hundred cases occurred among Russian soldiers in the Far East (3) during World War II. Several thousand cases of war nephritis, clinically similar to Nephropathia epidemica (NE), were reported among British soldiers stationed in Flanders during World War I (4), and about 16,000 cases of NE occurred among German soldiers in Lapland and prisoners in Yugoslavia during World War II (5). About 14,000 cases of war nephritis clinically similar to NE were described among Northern Armies in the American Civil War (6). In South Korea, 500 to 900 persons are hospitalized annually with this disease and about one third of them are soldiers. There were about 114,000 cases of HFRS in China in 1986 with 7% mortality, and several hundred cases of HFRS occurred in other countries of Asia and Europe (7). The causative agent was first discovered in 1976 from *Apodemus* mice (8) and isolated from patients in 1978 (9). The etiologic agent of KHF has been propagated in a human cell culture line (10), and it was named Hantaan virus after the Hantaan river which runs along the 38th Parallel between South and North Korea (11). Antigenic, genetic properties and EM findings indicated that Hantavirus is a new genus of Bunyaviridae (12,13,14,15). A close etiological relationship was established between KHF and HFRS in USSR, NE in Scandinavia and EHF in Eastern Europe, Japan and China (9,16,17,18). The working group on HFRS at a WHO meeting in Tokyo, 1982 recommended that the above mentioned diseases with different names should be referred to as "Haemorrhagic Fever with Renal Syndrome (HFRS)"(7). Recent sero-epidemiologic surveys showed that Hantaviruses are ubiquitous in the world. Antibodies against Hantaan virus in human sera were demonstrated in India, Thailand, Iran, Greece, U.S., Canada, Bolivia, Brazil, Gabon and Republic of Central Africa (19,20, 21,22,23) and recently in Taiwan, Philippine, Malaysia, Singapore, Hong Kong, Fiji, Hawaii, Argentine, Uruguay and Paraguay (24). Intraspecific transmission of Hantaan virus in *Apodemus* mice (25) was shown and infection occurred among cage-mates up to 360 days after infection, while large amounts of virus were excreted in urine and saliva, and no evidence for the participation of ectoparasites in virus transmission was obtained. Infection with Hantaan virus is thought to be silent in animals (26), but is associated with diverse clinical symptoms in man (27). A severe form is common in east Asia, while most European cases are mild. It usually produces sporadic disease, but under

special circumstances epidemics occur. Although predominantly associated with rural areas, it is now being recognized as an urban problem in some countries (28,29) and a particular hazard to laboratory staff using rodents for biomedical research (30,31, 32,33,34). From 1975 to 1983, 129 cases of HFRS, of which one was fatal, occurred in 22 animal rooms of research Institutes in Korea and Japan among personnel of the animal rooms, 71% (Korea) and 40% (Japan) of the rats had antibodies to Hantaan virus. Commercial rabbits bought from breeding firms in Korea and Japan were seropositive to Hantaan virus and serum antibodies were found in 3.5% of 792 New Zealand rabbits (35). We have registered a Hantaan related virus isolated from an urban rat caught in Seoul in 1980 as Seoul virus in 1985 (36). Several strains of Seoul virus were isolated from urban rats caught in Korea and Japan (37) and many strains of Hantaan and Seoul virus were isolated from blood of HFRS patients in Vero E6 cell cultures (38). Recently, there have been several outbreaks of acute hemorrhagic diseases among soldiers and farmers before and during the epidemic season of HFRS in Korea and it was confirmed that leptospirosis, scrub typhus and other rickettsiosis are the hemorrhagic diseases existing in Korea (39). There are still many problems to be answered in research work of HFRS and some important issues are: a) global survey of Hantavirus infection and HFRS b) serologic identification of new hantaviruses isolated in the different parts of the world c) an animal model mimic to man d) pathogenesis of hemorrhages and nephritis and e) therapentic agents and vaccines. This report describes 1) seroepidemiologic surveys of HFRS and scrub typhus in Korea 2) identification of a new serotype of Hantavirus 3) global serologic surveys for the presence of Hantavirus infection and 4) isolation of Seoul virus from Syrian hamster and susceptibility of inbred hamsters to hantaviruses, the etiologic agents of HFRS.

MATERIALS AND METHODS

Survey areas

Survey areas for reservoir of HFRS and isolation of Hantaan and related agents from field mice, laboratory rats and urban rats were Wuncheon and Jinhae, Korea and Argentina. Frozen lungs and sera from rodents from Argentina were shipped in dry ice to Seoul by Air Flight.

Collection of field and urban rodents

Field and house rodents were captured by means of baited live traps and normal *Apodemus* mice were captured on Jeju island as described (9.29). Seronegative *Apodemus* mice and Wistar rats were used as sensitive detectors for Hantavirus isolation.

Processing rodents

Living rodents were identified and bled by cardiac puncture under chloroform anesthesia. Serum was separated for antibody titration. Necropsy tissue include lungs and kidneys. A portion of each organ was examined immediately by IFA for Hantavirus antigen and the remaining portion were frozen at -70°C until processing for virus isolation.

Specimens from patients

Sera collected from suspected HFRS patients were used for serodiagnosis. Larger amounts of hyperimmune convalescent serum was collected from HFRS patients for experimental use.

Hantaviruses

All experimental and diagnostic work were done with Vero E-6 and A549 cells infected with Hantaan virus, strains 76/118 and ROK83/109 isolated from patient bloods and adapted in Vero E-6 cells. To titrate the virus from rat lungs, 10% lung suspensions are prepared with BSS containing 0.2% bovine albumin clarified at 5,000 G for 20 min. at 4°C and supernatants are used as inoculum. The ID50 of strains 76/118 and 83/109 in Vero E6 cells is $10^{5.3}$, $10^{6.2}$ and ID_{50} of strains 80/39 and Sotkamo in Vero E6 cells is $10^{5.8}$ and $10^{4.2}$/1.0 ml, respectively. All strains of hantaviruses are free from reovirus. It was proved by FA staining with polyvalent anti-reovirus immune sera and by antibody responses in rabbits and rats after inoculation of Hantaan and Seoul virus I.M.

Preparation of antisera

In addition to convalescent sera obtained from HFRS patients and antisera from naturally infected rats and mice, laboratory animals were used as a source of antibody. Sera from immunized rabbits and rats were employed.

Tissue culture cells

A-549 (10) and Vero E6 cells (12) were grown as described previously and used for virus isolation, preparation of FA antigen and virus plaque assay.

Virus isolation

The details of techniques used for demonstration of Hantavirus antigen by IFAT and virus isolation from HFRS patients and animals in Vero E6 cell cultures and in animals have been described previously (9,31,33,35).

Demonstration of antigen and antibodies of HFRS by use of immunofluorescent antibody techniques (IFAT)

The techniques employed for demonstration of antibodies and antigens of Hantavirus in specimens from patients, rodents and other animals have been described in detail (9,29,35).

Plaque reduction neutralization test (PRNT)

Neutralizing antibody titers were determined by plaque reduction methods employing immunoperoxidase straining (40). Hantaan and Seoul virus plaques developed readily in 5 to 7 days but Puumala and Prospect Hill virus plaques developed in 7 to 10 days under 0.5% methycellulose. PRNT titers are expressed as the reciprocal of the highest dilution of serum resulting in 80% or greater reduction in the number of virus plaques.

ELISA test

This test for demonstration of IgG and IgM antibodies against Hantaan virus was developed at USAMRIID. However, it can not differentiate Hantaan virus infection from Seoul virus infection because of cross reaction between them and the method is as described (41).

RESULTS

A. Seroepidemiological survey of HFRS and rickettsiosis in Korea in 1978.

1. Epidemiologic features of HFRS

There were 701 hospitalized cases of HFRS confirmed serologically at our Institute in 1987 and 5 of them were US Army soldiers as shown in Table 1. Total no. of serum from suspected HFRS in 1987 examined against Hantaan virus was 2,311 and only about 31% of them was HFRS patients as shown in Table 2. The ratios of serologically confirmed HFRS patients among clinically suspected HFRS patients by civilian doctors and ROK Army and US Army doctors are about 26%, 77% and 12%, respectively as shown in Tables 3 and 4. It is noteworthy that ratio of confirmed cases to suspected cases in 1980s is lower than that of 1970s because clinicians have sent us sera from only severe cases suspect of HFRS in 1970s while they are sending us more sera from mild suspected HFRS patients in these years. Clinicians have made better clinical diagnosis of HFRS during the epidemic season, October - January, than non-epidemic season of HFRS. Monthly occurrence of HFRS among civilians, ROK Army and US Army in 1987 is shown in Table 4, and these figures from 1966 to 1987 were compared in Table 5. Patients occur throughout the year and there are two peaks, a small peak in June and July, and a large peak in October - January. One of the new epidemiologic features of HFRS in Korea is increasing number of HFRS patients in urban areas of large cities as shown in Table 6. There were about 100 cases of HFRS in Seoul, Incheon and Pusan cities in 1987. These patients were only hospitalized severe cases, and usually moderate and mild cases are not included because Seoul virus infection is mild and usually diagnosed clinically as influenza and hepatitis. HFRS cases occurred in all district of Seoul as shown in Table 7. Recent findings show that there are an increasing no. of HFRS among children, and male patients are dominant group of HFRS as shown in Table 8 although 163 male soldier patients were not included in this no. of male cases. Table 9 shows occurrence of HFRS among ROK soldiers in different locations, and that about 90% of the patients were in Kyunggido and Kangwondo where main ROK armed forces are stationed. All of 5 HFRS patients among U.S. Army soldiers occurred in Kyunggido, where the 2nd Division of U.S. Army is stationed.

2. Epidemic outbreak of rickettsiosis before and during the epidemic season of HFRS in 1987.

As shown in Tables 2, 10 and 11, total no. of confirmed cases of scrub typhus was 209 among 1,530 HFRS suspected sera tested. These sera from the hospitalized patients were sent to our laboratory from hospitals in and nearby cities of Seoul for serologic diagnosis of HFRS. The no. of scrub typhus patients among civilians ROK Army and U.S. Army is 199, 8 and 2 respectively. Almost all of the patients

Table 1.
Hospitalized cases of Hemorrhagic fever with renal syndrome patients in the Republic of Korea.

Year	Korean civilian	Korean soldiers	US soldiers	Total
1951	...	...	827	827
1952	...	...	833	833
1953	...	...	455	455
1954	19	...	307	326
1955	...	...	20	20
1956	...	26	28	54
1957	...	21	13	34
1958	...	20	15	35
1959	...	47	79	126
1960	...	185	10	195
1961	...	341	27	368
1962	...	311	29	340
1963	...	257	11	268
1964	18	205	22	245
1965	2	110	99	211
1966	11	82	36	129
1967	13	86	31	130
1968	26	102	28	156
1969	48	134	9	191
1970	131	221	13	365
1971	391	358	2	751
1972	186	203	0	389
1973	241	237	0	478
1974	176	251	0	427
1975	466	370	1	837
1976	585	304	4	893
1977	288	241	7	536
1978	207	168	10	385
1979	241	122	1	364
1980	185	72	1	258
1981	377	164	2	543
1982	378	123	3	504
1983	402	98	3	503
1984	568	156	6	730
1985	531	159	7	697
1986	530	166	14	710
1987	533	163	5	701
Total	6,553	5,503	2,958	15,014

Numbers of patients since 1978 are serologically confirmed cases at The Institute for Viral Diseases, Korea University.

Table 2.
Number of HFRS, leptospirosis and scrub typhus diagnosed serologically at The Institute for Viral Diseases, Korea University in 1987.

Korean	$\frac{\text{Total no. of HFRS}}{\text{Total no. of serum tested}}$ ѱ	=	$\frac{696}{2,268}$ (31 %)
	$\frac{\text{Total no. of leptospirosis}}{\text{Total no. of serum tested}}$	=	N.T.
	$\frac{\text{Total no. of scrub typhus}}{\text{Total no. of serum tested}}$	=	$\frac{207}{1,500}$ (14 %)
US Army	$\frac{\text{Total no. of HFRS}}{\text{Total no. of serum tested}}$	=	$\frac{5}{43}$ (12 %)
	$\frac{\text{Total no. of leptospirosis}}{\text{Total no. of serum tested}}$	=	$\frac{0}{4}$ (0 %)
	$\frac{\text{Total no. of scrub typhus}}{\text{Total no. of serum tested}}$	=	$\frac{2}{30}$ (7 %)

ѱ : $\frac{\text{No. of serologically confirmed patient}}{\text{No. of suspected patient tested}}$

Table 3.
Number of serologically confirmed HFRS patient among clinically suspected HFRS patients at The Institute for Viral Diseases, Korea University from 1978 to 1987.

Year	Civilian (%)	ROK Army	US Army	Total
1978	207/221* (93.6%)	168/184 (91.3%)	10/29 (34.4%)	385/434 (89%)
1979	241/305 (79.0%)	122/145 (84.1%)	1/11 (9.0%)	364/461 (79%)
1980	185/386 (47.9%)	72/75 (96.0%)	1/3 (33.3%)	258/464 (56%)
1981	377/688 (54.7%)	164/203 (80.7%)	2/2 (100%)	345/893 (61%)
1982	378/901 (41.9%)	123/155 (79.3%)	3/13 (23.0%)	504/1,069 (48%)
1983	402/889 (45.2%)	98/154 (63.6%)	3/12 (25.0%)	503/1,055 (48%)
1984	568/1,256 (45.2%)	156/215 (72.5%)	6/12 (50%)	730/1,483 (50%)
1985	531/1,779 (29.8%)	159/344 (46.2%)	7/21 (33.3%)	697/2,144 (33%)
1986	530/1,765 (30.0%)	166/281 (59.0%)	10/38 (26.3%)	706/2,084 (34%)
1987	533/2,057 (25.9%)	163/211 (77.2%)	5/43 (11.6%)	701/2,311 (31%)
Total	3,952/10,247 (39%)	1,391/1,967 (71%)	48/184 (26%)	5,193/12,393 (42%)

* $\frac{\text{No. of positive serum}}{\text{No. of serum tested}}$ (%)

Table 4.
Number of serologically confirmed cases of Hemorrhagic fever with renal syndrome patients at The Institute of Viral Diseases, Korea University in Korea in 1987.

Month	No. of antibody positive sera against Hantaan virus / No. of tested sera from suspected patients			
	Civilian	ROK Army	US Army	Total
1	30/124	9/14	0/4	39/142
2	5/71	4/5	0/2	9/78
3	9/84	1/5	0/5	10/94
4	12/82	2/3	0/3	14/88
5	23/117	2/3	n.t.	25/120
6	11/89	10/11	n.t.	21/100
7	33/100	7/10	0/1	40/111
8	13/136	1/5	0/2	14/143
9	21/222	2/4	0/2	23/228
10	86/429	36/50	1/9	123/488
11	175/373	52/59	3/12	230/444
12	115/273	37/42	1/2	153/317
Total	533/2,100 (25.4 %)	163/211 (77.3 %)	5/42 (12 %)	701/2,353 (30 %)

Table 5.
Monthly incidence of Hemorrhagic fever with renal syndrome patients in the Republic of Korea, 1966 - 1987.

Year	Month												Total
	1	2	3	4	5	6	7	8	9	10	11	12	
1966	2	3	3	1	4	9	6	2	1	16	56	26	129
1967	2	1	0	1	4	10	2	4	8	29	50	19	130
1968	3	1	0	4	7	9	7	6	8	40	50	21	156
1969	4	0	4	1	8	12	7	8	5	41	66	35	191
1970	1	0	0	1	6	9	8	1	15	58	154	112	365
1971	13	1	2	7	14	23	13	19	33	140	348	148	761
1972	15	5	5	12	17	27	16	10	18	80	142	42	389
1973	12	3	3	4	6	10	11	13	19	117	211	69	478
1974	11	0	1	7	17	13	13	10	19	113	151	72	427
1975	25	5	3	3	8	32	22	22	27	177	360	153	837
1976	40	12	5	11	12	36	46	33	111	156	319	112	893
1977	7	0	0	2	8	57	21	19	29	93	226	74	536
1978	17	8	2	2	11	10	11	9	9	78	156	93	406
1979	12	4	6	7	21	16	21	12	9	79	124	53	364
1980	19	6	4	8	14	11	5	5	6	40	74	66	258
1981	12	7	1	4	4	17	21	6	15	80	233	143	543
1982	44	11	10	9	15	13	16	15	15	79	178	99	504
1983	34	7	2	5	9	16	16	3	13	60	186	152	503
1984	35	7	8	10	13	24	12	10	13	125	304	169	730
1985	45	18	12	8	21	32	21	21	12	74	254	181	699
1986	46	11	8	19	22	24	24	14	25	114	213	186	706
1987	39	9	10	14	25	21	40	14	23	123	230	153	701
Total	438	119	89	140	266	431	359	256	433	1,912	4,085	2,178	10,706

Table 6.
Distribution of confirmed cases of HFRS in Korea in 1987 at The Institute for Viral Diseases, Korea University (Civilian).

Name of Province	Month												Total
	1	2	3	4	5	6	7	8	9	10	11	12	
Seoul city	6	0	3	5	6	3	2	6	1	10	18	18	78
Incheon city	1	1	3	0	1	0	0	1	0	0	4	3	14
Pusan city	2	0	0	0	0	1	0	0	0	1	0	0	4
Kyounggi-do	14	2	2	6	8	2	15	0	6	29	53	34	171
Kangwon-do	2	0	0	0	2	1	3	3	3	14	11	6	45
Chungcheongbuk-do	1	0	1	1	2	0	0	1	0	6	19	13	44
Chungcheongnam-do	4	1	0	0	0	1	4	2	4	8	41	26	91
Jeollabuk-do	0	0	0	0	2	1	2	0	3	4	6	1	19
Jeollanam-do	0	0	0	0	0	0	1	0	3	2	5	4	15
Kyoungsangbuk-do	0	0	0	0	2	2	4	0	0	11	17	8	44
Kyoungsangnam-do	0	1	0	0	0	0	2	0	1	1	1	2	8
Total	30	5	9	12	23	11	33	13	21	86	175	115	533

Table 7.
Distribution of HFRS patients in districts of Seoul city in 1987.

Name of district	No. of patient
Seongbuk-ku	2
Kwanak-ku	0
Kangdong-ku	11
Dobong-ku	5
Seongdong-ku	2
Kangnam-ku	6
Mapo-ku	5
Kangseo-ku	6
Joong-ku	3
Youngdeungpo-ku	3
Dongjak-ku	1
Dongdaemun-ku	14
Seodaemun-ku	4
Kuro-ku	13
Yongsan-ku	1
Jongro-ku	0
Eunpyung-ku	2
Total	78

Table 8.
Age and sex distribution of HFRS, leptospirosis and scrub typhus in Korea, 1987.

Age	HFRS			leptospirosis			scrub typhus		
	M	F	Total	M	F	Total	M	F	Total
0 - 10	1	1	2	n.t.	n.t.	n.t.	0	0	0
11 - 20	28	13	41	n.t.	n.t.	n.t.	6	3	9
21 - 30	69	16	85	n.t.	n.t.	n.t.	26	7	33
31 - 40	90	30	120	n.t.	n.t.	n.t.	13	11	24
41 - 50	97	32	129	n.t.	n.t.	n.t.	21	11	32
51 - 60	55	43	98	n.t.	n.t.	n.t.	22	20	42
> 61	20	38	58	n.t.	n.t.	n.t.	24	25	49
Total	360 (68%)	173 (32%)	533 (100%)	n.t.	n.t.	n.t.	112 (59%)	77 (41%)	189 (100%)

Table 9.
Occurence of HFRS patients among ROKA soldiers in different areas of Korea in 1987.

Name of area	No.of patient	Name of area	No.of patient
Seoul city	2	Kwangju	1
Kyunggido		Pucheon	1
Paju	41	Janggok	1
Pocheon	16	Dongducheon	1
Kimpo	12	Kangwondo	
Yeoncheon	23	Chulwon	23
Yangju	6	Whacheon	11
Euijeongbu	6	Inje	2
Yangpyung	4	Koseong	1
Koyang	2	Hongcheon	1
Suwon	1	Yangku	1
Kapyung	1	Chungcheongnamdo	
Ilsan	1	Nonsan	2
Dukjeong	1	Chungcheongbukdo	
Seongnam	1	Goeseong	1

Total: 163 patients

Table 10.
Number of confirmed hospitalized cases of HFRS and scrub typhus among civilian at the Institute of Viral Diseases, Korea University in Korea, 1987.

Month	HFRS			scrub typhus		
	M	F	Total	M	F	Total
1	27/85[1]	3/39	30/124	0/87	1/24	1/111
2	4/47	1/24	5/71	0/47	0/24	0/71
3	6/55	3/29	9/84	0/55	0/29	0/84
4	9/50	3/32	12/82	0/15	0/10	0/25
5	13/78	10/39	23/117	0/18	0/7	0/25
6	11/61	0/28	11/89	0/7	0/5	0/12
7	28/78	5/22	33/100	3/24	0/4	3/28
8	9/91	4/45	13/136	6/91	4/45	10/136
9	11/156	10/66	21/222	33/156	13/66	46/222
10	54/246	32/183	86/429	58/246	60/183	118/429
11	117/200	58/172	175/373	6/19	8/16	14/35
12	76/146	39/87	115/233	6/146	1/87	7/233
Total	365/1293	168/767	533/2060	112/911	87/500	199/1411

[1] : $\frac{\text{No. of serologically confirmed patient}}{\text{No. of suspected patient tested}}$

Table 11.
Number of confirmed hospitalized cases of HFRS, scrub typhus among ROK soldiers at The Institute of Viral Diseases, Korea University in Korea, 1987.

Month	HFRS	scrub typhus
1	9/14[a]	0/8
2	4/5	n.t.
3	1/5	0/2
4	2/3	n.t.
5	2/3	n.t.
6	10/11	0/1
7	7/10	2/2
8	1/5	0/4
9	2/4	1/4
10	36/50	3/50
11	52/59	2/18
12	37/42	n.t.
Total	163/211	8/89

[a] : $\frac{\text{No. of serologically confirmed patient}}{\text{No. of suspected patient tested}}$

Table 12.
Number of confirmed hospitalized cases of HFRS, leptospirosis and scrub typhus among US army soldiers at The Institute of Viral Diseases, Korea University in Korea, 1987.

Month	HFRS	leptospirosis	scrub typhus
1	0/4 1/	n.t.	0/4
2	0/2	n.t.	0/2
3	0/5	0/3	0/5
4	0/3	0/1	0/3
5	n.t.	n.t.	n.t.
6	n.t.	n.t.	n.t.
7	0/1	n.t.	0/1
8	0/2	n.t.	0/2
9	0/2	n.t.	0/2
10	1/9	n.t.	2/9
11	3/12	n.t.	0/2
12	1/2	n.t.	n.t.
Total	5/42	0/4	2/30

1/ : $\frac{\text{No. of serologically confirmed patient}}{\text{No. of suspected patient tested}}$

Table 13.
Distribution of confirmed cases of scrub typhus in Korea in 1987 at The Institute for Viral Diseases, Korea University (Civilian).

Name of Province	Month												Total
	1	2	3	4	5	6	7	8	9	10	11	12	
Seoul city	1	0	0	0	0	0	0	3	3	25	2	2	36
Incheon city	0	0	0	0	0	0	0	0	0	2	0	0	2
Pusan city	0	0	0	0	0	0	0	0	0	1	2	0	3
Kyounggi-do	0	0	0	0	0	0	1	3	19	29	4	0	56
Kangwon-do	0	0	0	0	0	0	0	1	3	12	0	0	16
Chungcheongbuk-do	0	0	0	0	0	0	0	0	1	4	1	1	7
Chungcheongnam-do	0	0	0	0	0	0	1	1	9	31	0	3	45
Jeollabuk-do	0	0	0	0	0	0	0	0	1	1	1	0	3
Jeollanam-do	0	0	0	0	0	0	0	0	10	8	3	1	22
Kyoungsangbuk-do	0	0	0	0	0	0	0	1	0	3	1	0	5
Kyoungsangnam-do	0	0	0	0	0	0	1	1	0	2	0	0	4
Total	1	0	0	0	0	0	3	10	46	118	14	7	199

accurred during August-November, about a month before the large epidemic season of HFRS as shown in Table 10, 11 and 12. Distribution of scrub typhus patients in South Korea is shown in Table 13 and large portion of the patients were confirmed in Kyunggido, Chungcheongnamdo, Seoul city and Jeollanamdo, and some patients also occurred in other provinces as well. About 60% of scrub typhus patients was male and about a half of the patients was in age group of over 51 as shown in Table 8.

3. Seroepidemiologic survey of Apodemus agrarius, the reservoir of Hantavirus, Leptospira and R. tsutsugamushi, collected in the ROK Army and U.S. Marine camps in 1987.

We have carried out seroepidemiologic survey of Apodemus mice against Hantaan virus, *Leptospira interogans* and *R. tsutsugamushi* collected in the ROK Army and U.S. Marine camps, Wuncheon Kyounggido where outbreak of HFRS occurred among U.S. Marine in the fall of 1986 during "Bear-Hunt" exercise of U.S. Marine according to the request of the U.S. Marine in Okinawa since we have demonstrated that *Apodemus agrarius* is the main reservoir host of the etiologic agents of acute hemorrhagic diseases in Korea in 1986 as we have described in my 1987 Annual Report (39). The results of survey are shown in Table 14 and in Figure 1. The rate of infected *Apodemus* mice with Hantaan virus in the U.S. Marine and ROK Army camps was 16% and 9% respectively, and the infection rate of *Apodemus* mice with leptospira in the U.S. Marine and ROK Army camps was 19% and 11%, respectively. Infection rate of *Apodemus* mice with *R. tsutsugamushi* in both U.S. Marine and ROK Army camps was about 32%. Infection rates of Apodemus mice with these 3 agents in Sachangri and Sangdong that are located about 4 Km and 8 Km away from the U.S. Marine camp are almost same as in the US Marine camp. It is noteworthy that infection rates of Apodemus mice living in the U.S. Marine camp with Hantaan virus and leptospira are much higher than Apodemus mice in the ROK Army camp. Seasonal infection rate of Apodemus mice with these 3 agents in both Army camps is shown in Figure 1. The peak of infection rate of *Apodemus* mice with *R. tsutsugamushi* was May (85%), and high infection rate with leptospira was September, and high infection rate of Hantaan virus was July and November.

4. Isolation of Hantaan virus from Apodemus agrarius caught in Jinhae, Kyungsangnamdo in the south coast of Korea in 1987.

Four strains of Hantaan virus were isolated from lungs of *Apodemus agrarius* in Vero E6 cells captured in rural areas of Jinhae, harbour of the ROK Navy where HFRS patients were not reported up to date but outbreaks of scrub typhus were confirmed recently. These viruses were Hantaan virus by PRNT and monoclonal antibody assay and 2 strains of these viruses were sent to Dr. J. Dalrymple, USAMRIID for further molecular biological characterization.

Table 14.
Seroepidemiologic survey of Apodemus agrarius infected with Hantavirus, Leptospira and Scrub typhus collected in/near the Army camp, Wuncheon, Pochunkun, Kyounggido, 1986 and 1987.

Date of collection	Location	No. of antibody positive / No. of tested animal: Hantavirus[1]	Scrub typhus[2]	Leptospira[3]
December 9 - 14 1986	field in front of ROK Army camp	0/46(0%)	30/46(65%)	3/46(7%)
February 9 - 14 1987	field beside ROK Army camp	7/39(18%)	20/39(51%)	0/39(0%)
March 16 - 21 1987	US Army camp	7/26(30%)	7/26(30%)	1/26(4%)
	ROK Army camp	1/25(4%)	3/25(12%)	1/25(4%)
April 6 - 11 1987	US Army camp	4/23(17%)	9/23(39%)	0/23(0%)
	ROK Army camp	1/24(4%)	0/24(42%)	0/24(0%)
May 11 - 16 1987	US Army camp	6/19(32%)	17/19(90%)	0/19(0%)
	ROK 628 camp	7/39(18%)	[illegible]/39(82%)	0/39(0%)
June 29 - July 4 1987	US Army camp	0/16(0%)	4/16(25%)	1/16(6%)
	ROK Army camp	1/22(5%)	7/22(32%)	0/22(0%)
July 28 - 31 1987	US Army camp	7/21(33%)	10/21(48%)	8/21(38%)
	ROK Army camp	0/24(0%)	5/24(21%)	5/24(21%)
	Sachangri	5/33(15%)	15/33(46%)	6/33(18%)
August 5 - 10 1987	US Army camp	5/17(29%)	2/17(12%)	4/17(24%)
	ROK Army camp	0/15(0%)	5/15(33%)	2/15(13%)
	Sang Dong	2/21(10%)	1/21(10%)	6/21(29%)
September 15 - 19 1987	US Army camp	1/25(4%)	5/25(25%)	11/25(44%)
	ROK Army camp	2/33(6%)	8/33(24%)	14/33(42%)
October 19 - 24 1987	US Army camp	3/32(9%)	[illegible]4(43%)	8/32(25%)
	ROK Army camp	6/31(19%)	1/5 (20%)	5/31(16%)
November 2 - 6	US Marine camp	5/19(26%)	n.t.	n.t.
23 - 28	US Army camp	1/31(3%)	2/31(7%)	4/34(13%)
	ROK Army camp	4/30(13%)	6/30(20%)	2/30(7%)
9 - 14	Chinhae	12/34(35%)	2/34(6%)	8/31(26%)
December 21 - 24	US Army camp	1/13(8%)	3/13(23%)	2/13(16%)
	ROK Army camp	0/14(0%)	1/14(8%)	2/14(15%)
Total Feb. - Dec. 1987	US Army camp	35/223(15.7%)	65/205(31.7%)	39/205(19.0%)
	ROK Army camp	22/296(7,4%)	88/270(32,6%)	31/296(10.5%)
	Sachangri & Sang Dong	12/73(16.4%)	16/54(29.6%)	12/54(22.2%)

[1] Hantaan virus, 76/118 strain
[2] R. tsutsugamushi, Kato strain
[3] L. interogans, mwogala strain

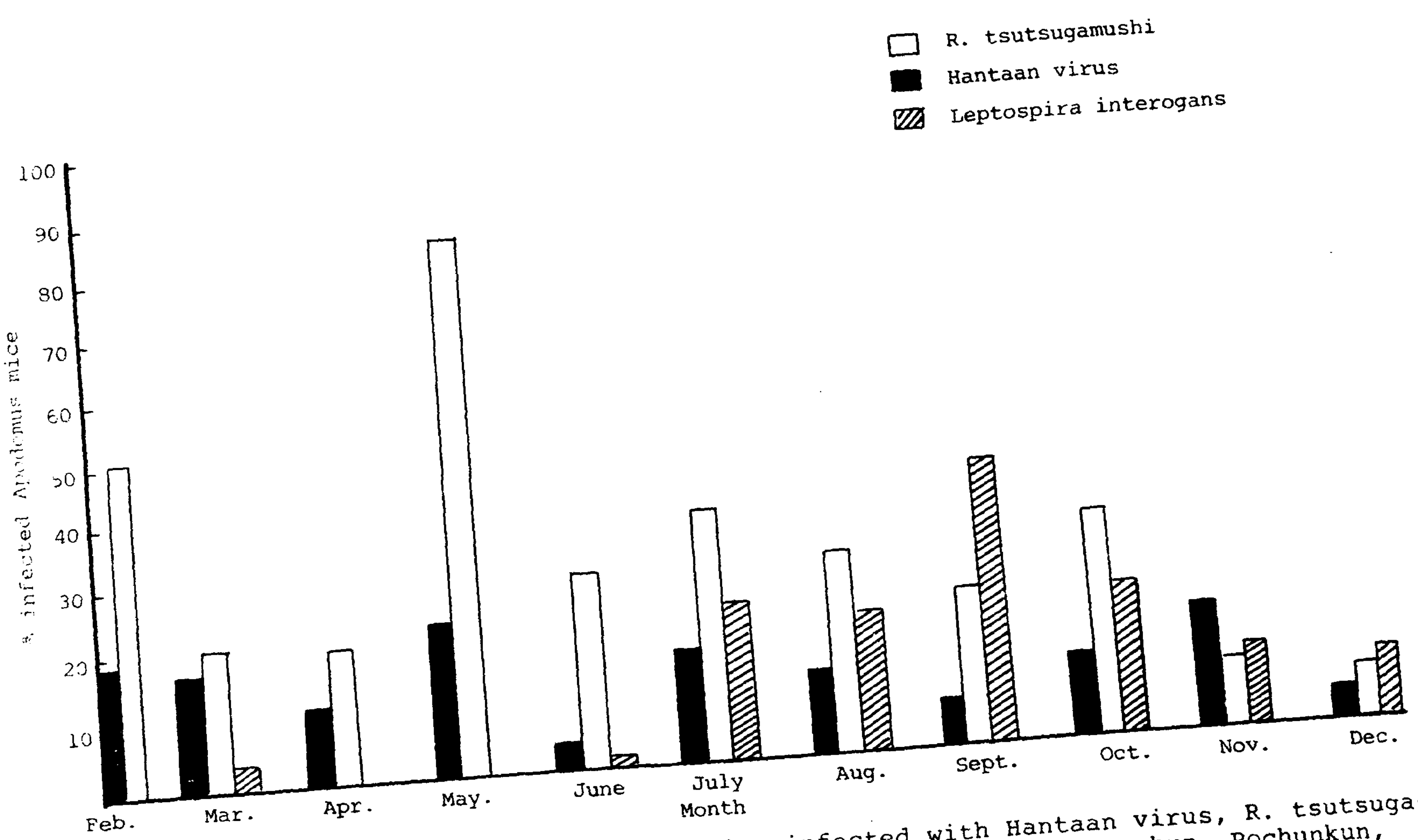


Fig. 1. Seroepidemiologic survey of field mice infected with Hantaan virus, R. tsutsugamushi and Leptospira interogans collected in/near the Army Camps, Wunchun, Pochunkun, Kyounggido, Korea in 1987.

B. Identification of a new serotype of Hantavirus.

Suspension of antigen positive lungs from *Apodemus agrarius* collected in Maaji, Kyunggido, Korea in 1980 was inoculated intramuscularly into laboratory-bred, seronegative striped field mice (*Apodemus agrarius coreae*), derived from animals captured in an HFRS-nonendemic region in Korea. Hantaan virus like antigen was detected in lung tissues of 3 of 3 mice 21 days postinoculation by IFAT. A 10% lung suspension was passaged once into colonized *Apodemus flavicollis*, following which the virus was adapted to Vero E6 cells. The virus isolate reacted with sera from patients with Korean hemorrhagic fever and with sera from patients of nephropathia epidemica by IFAT. Rat antiserum prepared against the isolate was tested by the PRNT to the prototype strains of the four established Hantavirus serotypes, namely, Hantaan virus strain 76/118, Seoul virus strain 80/39, Puumala virus strain Sotkamo, and Prospect Hill virus strain Prospect Hill-I.

As shown in the Table 15, sera from patients with Korean hemorrhagic fever and rat antiserum prepared against Hantaan virus strain 76/118 had high PRN antibody titers to the virus, indicating a close antigenic relationship between the new isolate and Hantaan virus. Sera from patients with *Rattus*-associated HFRS in Japan and rat antiserum prepared against Seoul virus strain 80/39 exhibited moderate cross-neutralization of the virus. PRN antibody titers of sera from nephropathia epidemica patients in Finland and Sweden and rat antiserum agianst Prospect Hill virus were low to this virus. In contrast, sera from HFRS patients in Listice and Banjaluka, Yugoslavia, and rat antiserum against the virus had high titers to the virus and exhibited moderate cross-neutralization of the heterologous serotypes.

The evidences show that this novel virus, while serologically related to Hantaan virus, is antigenically distinct and constitutes a new serotype. We named this virus Maaji virus after the name of village in Kyunggido, Korea where the *Apodemus* mice were caught.

C. Global serologic surveys for Hantavirus infection.

1. Hantavirus infection among laboratory personnel and general population in South America.

A serological survey was conducted to determine the prevalence of Hantavirus antibodies in the general population in Argentina, Uruguay, Paraguay and Bolivia, as well as among rodent-exposed laboratory workers in Argentina. Out of 748 individuals tested by IFAT, 20 proved positive for Hantaan virus 76/118 strain.of whom 15 also reacted against Seoul virus 80/39 strain and 2 against Puumala virus Sotkamo strain as shown in Table 16. Ten out of 72 Argentine laboratory workers were positive for either of the first 2 viruses by IFAT, ELISA, and/or plaque reduction neutralization test, in 4 of whom recent infection was demonstrated by IgM antibody presence. Four of them were infected with Seoul

Table 15.
Neutralization of Maaji virus (type 5) by sera from patients with Hemorrhagic fever with renal syndrome and rats immunized with Hantaviruses.

Serotype*	Patient and antisera	Reciprocal PRN titers to:			
		Maaji	76-118	80-39	Sotkamo
Hantaan	KHF	2,000	>2,000	NT	20
	KHF	>2,000	>2,000	200	20
	anti-83-138 serum	2,000	>2,000	NT	20
	anti-76-118 serum	2,000	>2,000	NT	20
Seoul	Japanese HFRS	>2,000	200	>2,000	200
	anti-Tchoupitoulas	640	160	>2,000	40
	anti-82-3 serum	640	20	2,000	20
	anti-80-39 serum	200	NT	>2,000	NT
Puumala	Finnish NE	20	20	20	>2,000
	Swedish NE	200	20	20	>2,000
	"	20	20	20	640
	"	40	20	NT	2,000
	Yugoslavian NE	40	40	20	>2,000
P.H.	anti-Prospect Hill-I	40	40	20	200
Maaji	Yugoslavian NE	2,000	20	NT	NT
	"	>2,000	200	NT	NT
	"	>2,000	40	NT	NT
	anti-Maaji serum	>2,000	2,000	200	200

* Serotypes were designated and classified on the basis of data on immunofluorescent and plaque reduction neutralization tests.

Table 16.
Antibody titers against Hantaviruses in 20 out of 748 sera from inhabitants of Argentina, Bolivia, Paraguay* and Uruguay, 1985-1986.

Origin of sera			Viral antigen and antibody titer					No. of positive/	(%) of
Country (Province)	Age	Sex	Hantaan		Seoul		Puumala	No. of sera	positive
			IFA	PRNA	IFA	PRNA	IFA	tested	sera
Argentina									
Jujuy	64	F	32		32		-	1/8	(12.5)
Salta	26	F	64		64		-	1/18	(5.6)
Chaco	43	M	64	20	256	20	64		
	52	F	64		-		-	3/39	(7.6)
	26	M	64		64		-		
Santiago del Estero	78	F	32		64		-		
	25	F	64		64		-	4/101	(4.0)
	30	F	128		128		-		
	50	F	32		32		-		
Tucuman	29	F	64		-		-	2/32	(6.2)
	38	F	128		128		-		
Misiones	32	F	64		64			1/10	(10.0)
Buenos Aires	54	M	128	20	256	20	64		
	23	F	128		-		-	4/401	(4.0)
	27	F	64		-		-		
	51	F	128		32		-		
Uruguay	48	M	32		32		-		
	34	F	64		64		-	2/205	(1.0)
Bolivia	34	F	32		32		-		
	17	F	128		128		-	2/28	(7.1)
Total			20		16		2	20/748*	(2.7)

* 29 sera from Paraguay and 177 from 10 other Argentine provinces were negative.

Table 17.

Antibody titers of 10 positive sera from 72 Argentine laboratory workers against Hantaan and Seoul viruses by IF, PRN and ELISA test, 1987.

Type of Job	Age	Sex	IF Ab titer (IgG)		PRN Ab titer		ELISA Ab titer HTNV		No.of positive/ No.of sera tested	(%) of positive sera
			HTNV	SEOV	HTNV	SEOV	IgG	IgM		
Professional	27	M	16	32	NT	NT	-	-		
Tissue culture technician	60	F	-	32	NT	NT	200	-		
Animal care-taker	40	M	64	64	NT	NT	200	-		
Animal care-taker	59	F	16	64	NT	NT	400	800	10/72	(13.9)
Professional	55	F	-	64	NT	NT	1600	400		
Animal care-taker	57	M	-	32	NT	NT	400	-		
Professional	43	M	256	NT	20	2000	25600	3200		
Technician	30	M	64	NT	20	200	6400	800		
Animal care-taker	35	M	128	NT	20	2000	15600	-		
Animal care-taker	50	M	128	NT	20	2000	12800	-		

PRN: Plaque reduction neutralization Ab: Antibody

HTNV: Hantaan virus SEOV: Seoul virus NT: Not tested

Table 18.
Seropositive rodents by IFAT against Hantaan virus in Argentina

Rodents	Place of origin	Species	No. of positive / Total samples	(%)
Laboratory rodents	Institution 1	Wistar rat	0/7	0.0
		Mus musculus	0/19	0.0
		Calomys musculinus	0/5	0.0
		Calomys laucha	0/6	0.0
		Calomys callidus	0/6	0.0
	Institution 2	Wistar rat	5/22	22.8
		Buffalo rat	2/23	8.4
	Institution 3	Wistar rat	3/5	60.0
		Akodon molinae	0/4	0.0
	Institution 4	Wistar rat	4/5	80.0
Urban rats	Buenos Aires	Rattus norvegicus	0/23	0.0
	Harbor	Rattus rattus	0/8	0.0
Wild rodents	Montes (Buenos Aires Province)	Calomys musculinus	3/8	37.5
		Akodon azarae	0/13	0.0
	La Pega (Mendoza Province)	Calomys musculinus	1/9	11.0

Table 19.
Antibody titers of laboratory and wild rodent sera from Argentina against Hantaviruses.

Origin of serum	Type of rodent and code no.	Antibody titers against virus		
		Hantaan		Seoul
		IFA	PRNT	PRNT
Laboratory rats Buenos Aires	Wistar-8	32	n.t.	n.t.
	Wistar-11	64	n.t.	n.t.
	Wistar-12	32	n.t.	n.t.
	Wistar-14	32	n.t.	n.t.
	Buffalo-22	32	n.t.	n.t.
	Buffalo-25	64	n.t.	n.t.
	Buffalo-29	64	n.t.	n.t.
Laboratory rats Mendoza	Wistar-58	32	n.t.	n.t.
	Wistar-59	64	< 20	20
	Wistar-62	1,024	< 20	20,000
Laboratory rats Mendoza	Wistar-63	1,024	20	20,000
	Wistar-64	32	< 20	200
	Wistar-65	2,048	< 20	2,000
	Wistar-67	64	< 20	< 20
Wild mice montes	C.musculinus-2	32	n.t.	n.t.
	C.musculinus-3	32	n.t.	n.t.
	C.musculinus-5	32	n.t.	n.t.
Mendoza	C.musculinus-7	32	n.t.	n.t.

virus or Seoul-like virus by PRNT as shown in Table 17.

Inapparent Hantavirus infection was thus demonstrated for the first time in 2.7% of inhabitants in Argentina and Uruguay, together with 13.9% among Argentine rodent-exposed laboratory workers.

2. Hantavirus infection in experimental and wild rodents in Argentina

Serum samples from rats, mice and voles in Argentina were collected and tested by IFAT and plaque reduction neutralization test to investigate the prevalence of anti-Hantavirus antibodies. One hundred and two sera were obtained from experimental rodents in 4 different animal-rooms of the 4 Institutes, 31 from urban rats in Buenos Aires and 30 from wild voles in 1986-1987 as shown in Table 18.

Anti-Hantavirus antibodies were detected in 22.5% of laboratory rats and in 13.3% of the vole *Calomys musculinus*, the main Junin virus reservoir, the etiological agent of Argentine hemorrhagic fever. Antibody titers of positive sera from rodents against Hantaan and Seoul viruses are shown in Table 19 and PRNT showed that laboratory rats were infected with Seoul virus as in Korea and Japan. Two out of 4 positive sera from *C. musculinus* contained IF antibodies against Prospect Hill virus and antibody titers of 2 sera were 64 and 32. Urban rats and laboratory mice and voles were found to be free of Hantavirus infection. The presence of Hantavirus infection is hereby reported for the first time in wild *C. musculinus* and in laboratory rats in Argentina.

D. Isolation of Seoul virus from Syrian hamster and susceptibility of inbred hamsters to Hantavirus, the etiologic agents of HFRS.

1. Isolation of Seoul virus from a Syrian hamster

A strain of Seoul virus was isolated from a Syrian hamster in both Wistar rats and Vero E6 cells directly. The hamsters were purchased from an animal supplier in Seoul in Summer of 1985 for leptospirosis research. Four hamsters among 7 Syrian hamsters purchased from the animal farm were seropositive against Hantaan virus and 1 of 4 positive hamsters was antigen positive by IFAT. Subsequently, a virus was isolated from the antigen positive lungs of the hamster in Vero E6 cells and this virus was classified serologically as Seoul virus by PRNT and monoclone assay. Table 20 shows the multiplication of the virus in lungs of Wistar rats and inbred hamsters LHC but *Apodemus agrarius* after inoculation of the 10% antigen positive lungs and kidney suspension intramuscularly. This is the first isolation of Hantavirus from naturally infected Syrian hamsters.

2. Attempts to develop an animal model in inbred hamsters for HFRS

Four strains of inbred Syrian hamsters (PD4, CB, LHC and LSH), Wistar rats and *Apodemus* mice were inoculated with Hantaan virus 76/118 strain and 83/109 strain, Seoul virus Egypt R/13120 strain and Puumala virus Sotkamo strain, and multiplication of the viruses and antibody formations after virus inoculation intramuscularly are shown in Tables 21-25. Hantaan virus 76/118 strain multiplied well and produced good antibodies in all of 4 different inbred hamsters as shown in Table 21. It was surprising to observe that 2 out of 4 PD4 hamsters and 1 out of 4 CB hamsters inoculated with Hantaan virus 76/118 strain died 19-21 days after virus inoculation as indicated in Table 21. Multiplication and specific viral antigen spots of Hantaan virus 76-118 in lungs and kidneys of the inbred CB hamster that died 19 days after virus inoculation was shown in Fig. 2.

Multiplication of Hantaan virus 76/118 in lungs and brain of inbred LSH hamster killed 23 days after virus inoculation was shown in Fig. 3. Specific immunofluorescent viral antigen spots were observed in the cytoplasm by IFAT. Courses of infection of inbred PD4 hamsters after inoculation of Hantaan virus 76/118 are shown in Fig. 4. IF antibody titers were 4,096 after 28 days and body weights of sick and dead hamsters were decreased significantly after 14 days after virus inoculation. Rectal temperatures of sick hamsters were not high compared with healthy hamsters. Albuminuria was demonstrated in the urine collected from PD4 hamsters only 12 and 16 days after inoculation of Hantaan virus 76/118 strain. Inbred PD4 hamsters were healthy and gained body weight after inoculation of Hantaan virus 83/109 strain and course of infection is shown in Fig. 5.

The results in this limited experiments shown that inbred hamsters supported multiplication of hantaviruses and some strain of Hantaan virus is pathogenic to PD4 and CB inbred hamsters after inoculation of the virus. Further experiments to confirm these results and evaluation of inbred hamsters as an useful animal model for HFRS are needed immediately.

Table 20.
Antibody response and multiplication of a Seoul virus isolated from a Syrian hamster in rodents after inoculation of the virus.

Route and dose of inoculum	Species of animal	Animal no.	IF antibody titers of serum on days after virus inoculation: 14	21	28	35	Presence of virus antigen in tissues on 35 days after virus inoculation: Lung	Kidney	Liver	Spleen
Seoul virus SNU/hamster 0.2 ml of 10% lung and kidney suspension of hamster, IM.	Apodemus agrarius	1	-	-	-	-	-	-	-	-
		2	-	-	-	-	-	-	-	-
		3	-	-	-	-	-	-	-	-
		4	-	-	-	-	-	-	-	-
	Wistar rat	1	-	256	4096	4096	+	-	-	-
		2		1024	4096	4096	+	-	-	-
		3	-	256	4096	4096	+++	-	+	+
		4	-	32	4096	4096	-	-	-	-
	Hamster LHC	1		-	-	-	-	-	-	-
		2	-	-	-	-	-	-	-	-
		3	-	-	-	1024	+++	+	++	+
		4	-	-	-	64	+++	+	-	-

Table 21.
Susceptibility of inbred hamsters to Hantaan virus (76/118) after inoculation of the virus intramuscularly.

Dose and route of inoculum	Inbred hamster	Animal no.	IF antibody titers on days 7	14	21	28	Virus antigens in tissues of hamsters on 31 days after virus inoculation: Lungs	Kidney	Liver	Spleen
Hantaan virus 76/118 Vero E6/passage 4	PD4	1	64	256	died(d-21)a/		++++	++	+	+
		2	16	256	1,024	4,096	++++	++	+	+
		3	32	256	1,024	4,096	++++	++	+	+
		4	16	256	died(d-19)a/		+++	++	+	+
	CB	1	32	256	1,024	1,024	+++	++	+	+
		2	64	256	1,024	4,096	++++	++	+	+
		3	-	256	1,024	4,096	+++	++	-	+
		4	16	256	died(d-19)a/		++++	++	+	+
	LHC	1	64	256	1,024	4,096	+++	++	+	+
		2	16	256	1,024	1,024	++	+++	+	+
		3	-	256	1,024	4,096	+++	+++	-	+
		4	16	256	1,024	4,096	++++	++	+	+
	LSH	1	16	256	1,024	4,096	++++	++	+	+
		2	16	256	1,024	4,096	+++	++	+	+
		3	64	256	1,024	4,096	++++	++	+	+
		4	16	256	1,024	4,096	+++	+++	+	+

a/ : Presence of Hantaan virus antigen in tissues of hamsters were tested when the animal died

Table 22.
Susceptibility of inbred hamsters to Hantaan virus (83/109) after inoculation of the virus intramuscularly.

Dose and route of inoculum	Inbred hamster	Animal no.	IF antibody titers on days 7	14	21	28	Virus antigens in tissues of hamsters on 31 days after virus inoculation: Lungs	Kidney	Liver	Spleen
Hantaan virus 83/109 Vero E6/passage 4	PD4	1	16	64	256	256	-	-	-	-
		2	-	256	1,024	4,096	+	+	-	-
		3	16	256	1,024	4,096	+	-	-	+
		4	16	64	1,024	killed(d-23)	-	-	-	+
	CB	1	-	64	256	killed(d-23)	-	-	-	-
		2	-	64	256	1,024	+	-	-	-
		3	16	16	256	1,024	-	-	-	-
		4	-	died(d-10)			-	-	-	-
	LHC	1	-	256	256	256	-	-	-	-
		2	64	256	1,024	4,096	-	-	-	-
		3	-	64	1,024	1,024	-	-	-	-
		4	64	256	1,024	killed(d-23)	+	-	-	-
	LSH	1	-	64	64	256	-	-	-	-
		2	16	256	1,024	4,096	-	-	-	-
		3	64	256	1,024	4,096	+	+	-	-
		4	-	64	1,024	killed(d-23)	+	-	-	+

Table 23.
Antibody response and multiplication of a Seoul virus isolated from a urban rat caught in Egypt in rodents after inoculation of the virus.

Route and dose of inoculum	Species of animal	Animal no.	IF antibody titers of serum on days after virus inoculation				Presence of virus antigen in tissues on 35 days after virus inoculation			
			14	21	28	35	Lung	Kidney	Liver	Spleen
Seoul virus Egypt R/13120, 0.2 ml of 10% lung suspension of rat, I.M.	Wister rat	1	256	4096	16384	16384	++	+	+	+
		2	-	1024	4096	4096	+++	-	+	+
		3	-	1024	8192	4096	++	-	+	+
		4	-	256	8192	4096	+	-	+	+
	Apodemus agrarius	1	-	-	-	-	-	-	-	-
		2	-	-	-	-	-	-	-	-
		3	-	-	-	-	-	-	-	-
		4	32	64	64	256	-	-	-	-
	Hamster LHC	1	32	32	64	256	++++	+	+++	-
		2	-	-	32	256	++++	++	+	+
		3	-	16	256	1024	++++	+	++	-
		4	32	256	4096	4096	++++	++	++	+

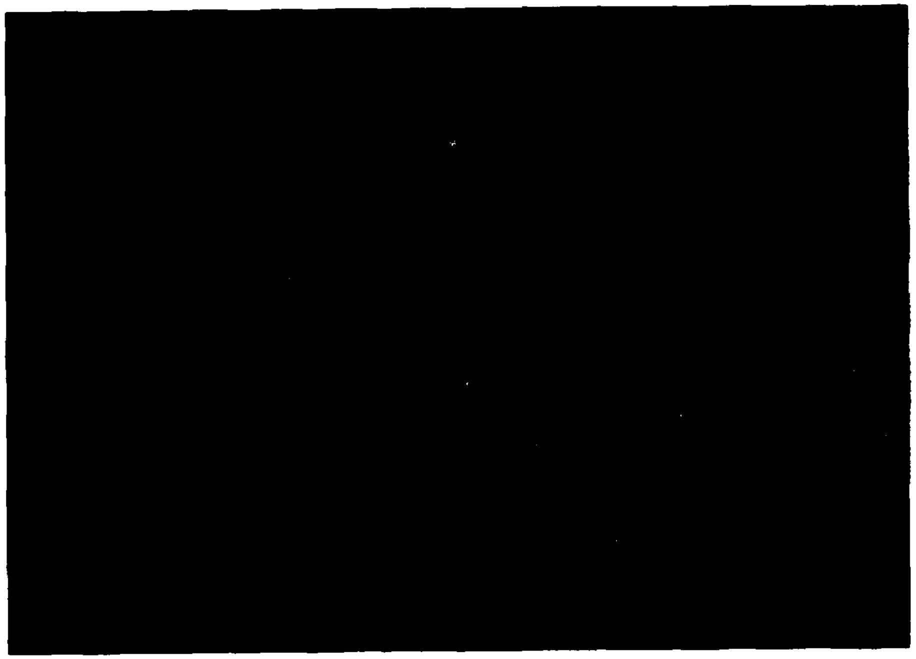
Table 24.
Antibody response and multiplication of Puumala virus (Sotkamo) in hamster and Apodemus agrarius after inoculation of the virus.

Route and dose of inoculum	Species of animal	Animal no.	IF antibody titers of serum on days after virus inoculation				Presence of virus antigens in tissues on 29 days after virus inoculation			
			7	14	21	29	Lung	Kidney	Liver	Spleen
Puumala virus Sotkamo, Vero E6/P4, 0.2ml	Apodemus agrarius	1	-	32	128	128	+	+	++	-
		2	-	64	256	512	-	-	-	-
		3	-	-	256	512	+	-	+	-
		4	-	32	128	128	-	-	-	-
	Hamster LHC	1	-	128	1,024	4,096	++++	+	++	-
		2	-	512	1,024	4,096	+++	+	++	+
		3	-	128	1,024	2,048	+++	+	++	+
		4	-	128	1,024	4,096	++++	+	++	+

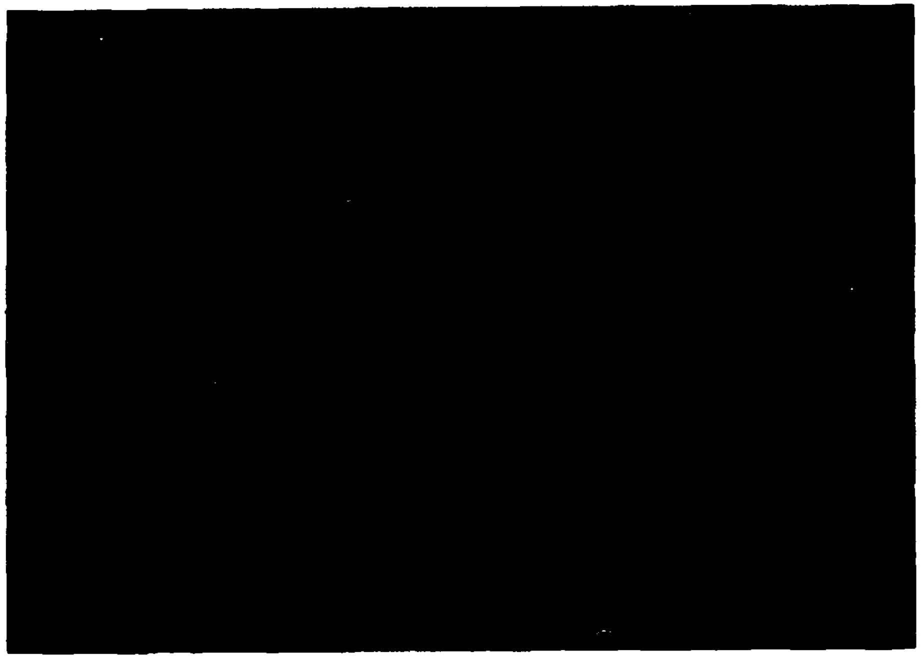
Table 25.
Cross reactivity of Puumala virus antisera from Apodemus mice and hamster against Hantaan virus, Seoul virus and Prospect Hill virus.

Puumala virus antiserum [a]	Animal no.	Virus antigen and IF antibody titers of sera bled on days after virus inoculation												
		Puumala virus				Hantaan virus			Seoul virus			P.H. virus		
		7	14	21	29	14	21	29	14	21	29	14	21	29
Apodemus mice antiserum	1	-	32	128	128	-	-	-	-	-	-	-	16	32
	2	-	64	256	512	-	-	-	-	-	-	-	128	128
	3	-	-	256	512	-	-	-	-	-	-	-	32	128
	4	-	32	128	128	-	-	-	-	-	-	-	-	16
Hamster LHC antiserum	1	-	128	1024	4096	-	32	128	-	32	256	-	64	256
	2	-	512	1024	4096	-	32	64	-	-	64	64	1024	1024
	3	-	128	1024	2048	-	32	256	-	-	64	-	256	512
	4	-	128	1024	4096	-	64	64	-	32	64	64	256	256

[a] Puumala virus antisera were made as described in Table 3.

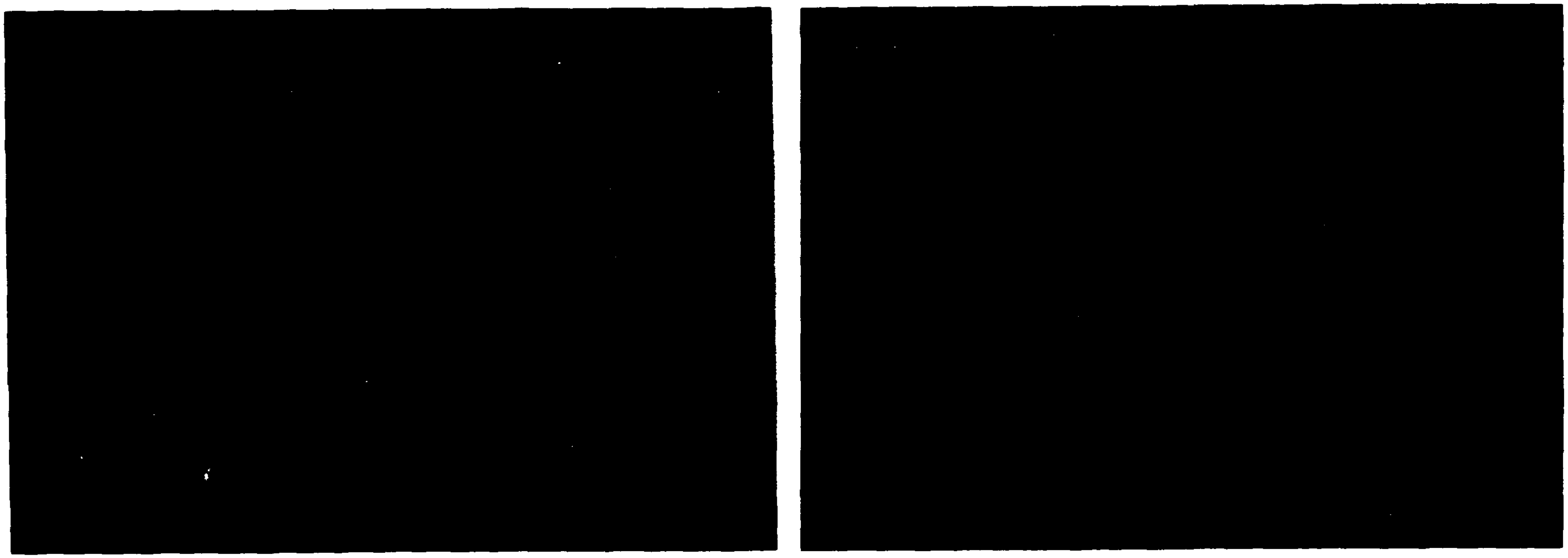


A (antigen ++++)



B (antigen ++)

Fig. 2. Lung (A) and kidney (B) sections of inbred hamster CB died 19 days after inoculation of Hantaan virus 76/118 strain (Ektachrome ASA 400, 2 min. 500X. Leitz SM-Lux). Specific immunofluorescent spotts of viral antigens were observed after reaction with rat anti-Hantaan virus serum by IFAT.



C (antigen ++++)

D (antigen ++'

Fig. 3. Lung (C) and brain (D) sections of inbred hamster LSH autopsied 23 days after inoculation of Hantaan virus 76/118 strain.

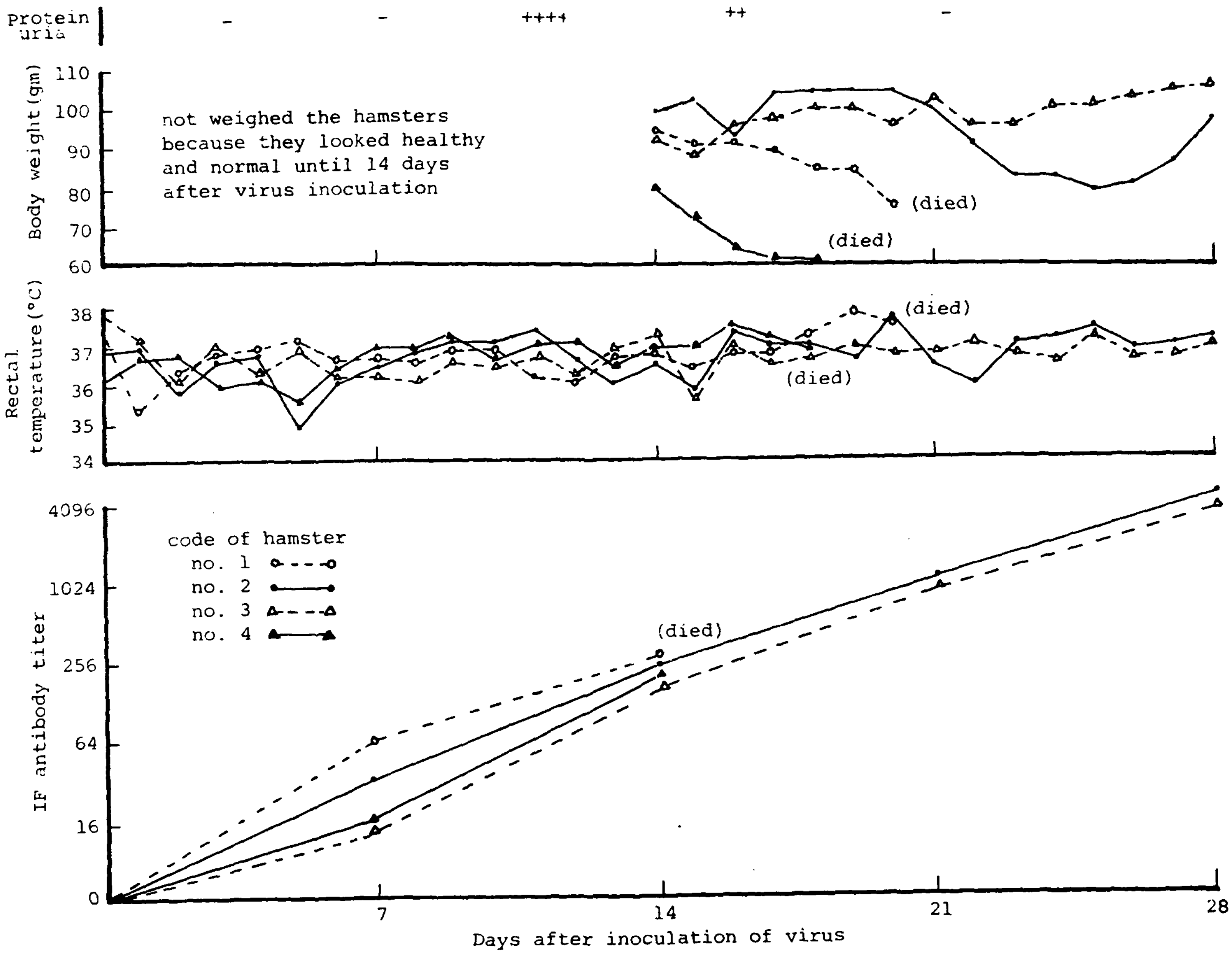


Fig. 4. Course of infection of inbred hamster PD4 after inoculation of Hantaan virus 76/118 intramuscularly.

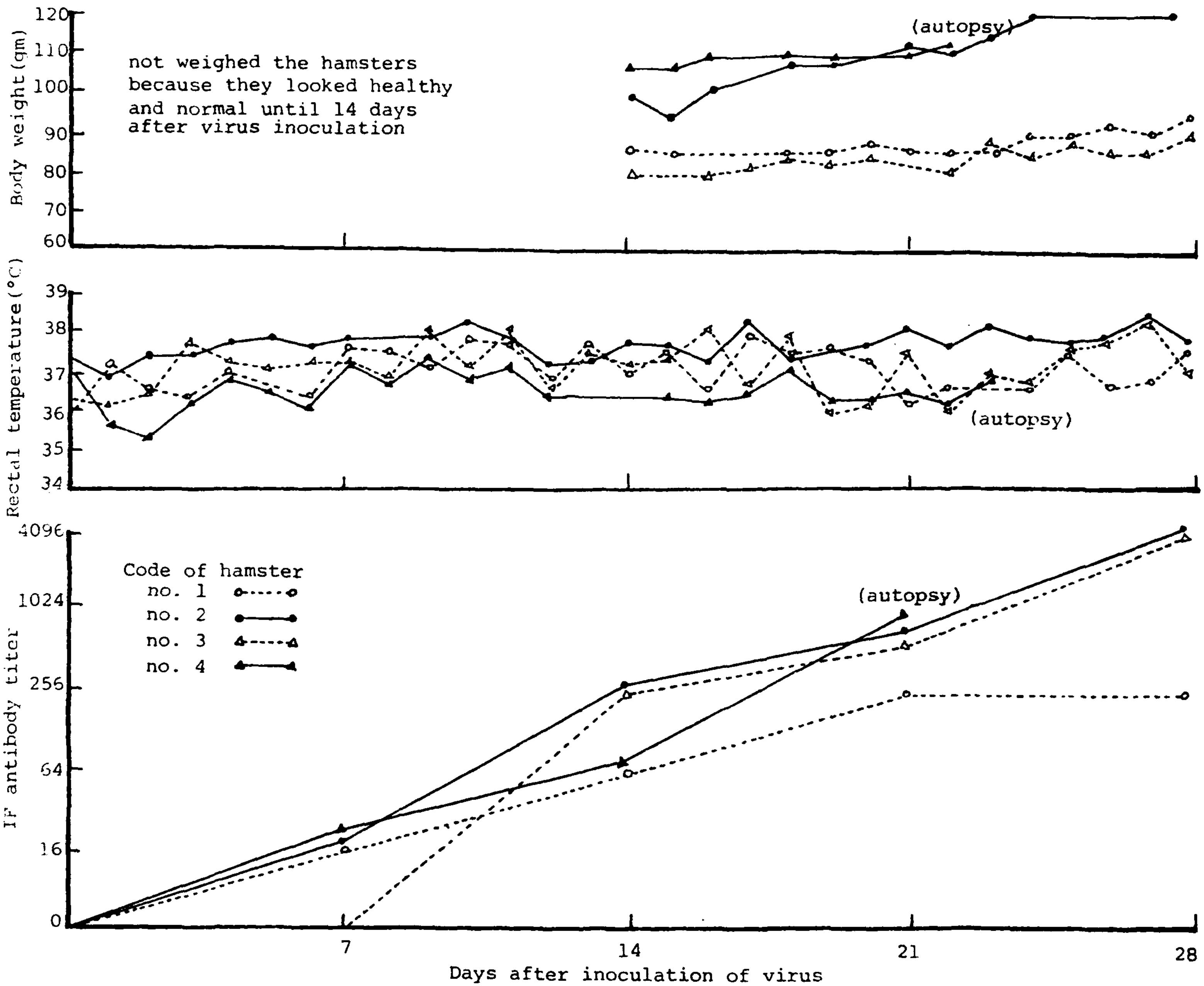


Fig. 5. Course of infection of inbred hamster PD4 after inoculation of Hantaan virus ROK83-109 intramuscularly.

DISCUSSION

Number of 701 cases of HFRS is only serologically confirmed no. of hospitalized patients at our Institute in 1987. Sera from suspect HFRS patients came from limited hospitals in and nearby cities of Seoul, therefore, real total no. of HFRS in entire South Korea should be at least three times more than no. of patients in table 1 because we might have examined only one third of HFRS cases according to the distribution of population and areas we covered since 1977.

It could be estimated that there are at least 2,000 cases of HFRS patient in S. Korea every year if serologic diagnostic capabilities are available at the endemic areas of S. Korea where we could not cover to make serodiagnosis. It is interesting to learn that no. of HFRS patient in urban areas of large cities is increasing recently and many cases occur in late fall from October to December, same epidemic season of HFRS in the rural endemic areas of Korea. More than 85% of total patients were confirmed in five Provinces located in northern parts of S. Korea as shown in table 6 but it might not mean that the Provinces are more heavily infected foci of HFRS than other Provinces since other Provinces are far from Seoul and it is very difficult to send sera from the suspect patients at acute stage of illness to our Institute for serologic diagnosis of the disease. All of the hospitals in endemic rural areas of Korea have incapability to make serologic diagnosis of HFRS although patients occur all over Korea except Jeju island. Distribution of occurrence of HFRS patient in Seoul is in all districts and every district had several cases of HFRS every year. It remains to be studied the risk factors and virulence of Hantavirus strains where about 10 million people are living and more than 10% of urban rats population is infected with Seoul virus (29). There is a large epidemic peak of HFRS in late fall and a minor peak in May-July every year and epidemic occurs among soldiers stationed in and near DMZ between South and North Korea. There were only 5 HFRS out of 43 suspect HFRS patients among about 40,000 U.S. soldiers stationed in Korea.

Recently, it has been known that clinical complex of acute hemorrhagic diseases in late summer and fall in Korea since 1982 is HFRS, leptospirosis and rickettsiosis as described in my 1987 annual report (45). A large outbreak of scrub typhus was confirmed from August to November and no. of patients was 207 (14%) among 1,530 suspect HFRS patients. We have not examined sera from suspect HFRS against leptospira this year because it is too much work and our Institute is mainly concerned with viral diseases. The evidences show that there are many scrub typhus and other diseases with unknown origin among suspect HFRS. Our preliminary screening tests with these sera from unknown fever patients indicate that there are murine typhus, other rickettsiosis and Colorado-tick fever like illness.

It was proved that Apodemus agrarius, the reservoir animal host of Hantaan virus, is the reservoir host of Leptospira interogans and R. tsutsugamushi as well in Korea (40). Apodemus agrarius is wide-spread in Eastern-Asia continent and most abundant field mice in Korea. There was an outbreak of HFRS among U.S. Marine during exercise "Bearhunt" in Wuncheon area, near DMZ in summer of 1986 (46) and 2 died out of 14 HFRS patients. It was requested by the U.S. Marine in Okinawa to investigate the infection rate of Apodemus agrarius with Hantaan virus in the U.S. Marine camp and other areas for prevention strategy of HFRS next year since exercise "Bearhunt" will be continued in this area in future. It is surprising to learn that Hantaan virus infection rate of Apodemus mice caught in US Army camp is about 16%, much higher than that of 9% in ROK camp although the two army camps are located side by side. However, infection rate of Apodemus mice with R. tsutsugamushi caught in U.S. Army and ROK Army camps is almost same, about 32%. This means that mode of transmission and maintenance of the two agents causing HFRS and scrub typhus are different in nature and much of the ecology of the two agents remains to be studied. Scrub typhus is known to be transmitted by trombicular mites (47) but HFRS is not considered as an arbovirus disease (25) though Kasahara et al (48) claimed that trombicular mites are vectors of HFRS in 1944. Still many issues of Hantavirus (3), such as biological characteristics of the virus in nature need to be investigated since Hantavirus is a new genus of Byunyaviridae and most of the viruses in this family are arboviruses.

Recent studies have demonstrated a near global distribution of Seoul virus among urban rats and the presence of this or other Hantaviruses among several different species and genera of small mammals (16-20,23). Clearly the genus Hantavirus (15) is widely distributed and maintained in a variety of different ecological settings. The degree to which Hantaviruses cause human disease, especially in areas where HFRS has not been traditionally recognized, is presently unknown. As WHO Collaborating Centre for Research on HFRS, we provided serological diagnosis for suspect HFRS in sera from throughout the world. In addition, we have collaborated with a number of investigators conducting small mammal surveys for evidence of Hantavirus infection and isolation of strains from urban rats. Results of these preliminary studies indicate that human disease due to Hantavirus is present in several areas where HFRS had not been previously diagnosed.

We demonstrated for the first time, serological evidence of human inapparent infection with Hantavirus in the general population of Argentina and Uruguay, and in laboratory workers of Argentina. However in Bolivia, antibodies against Hantaan virus had already been reported in human serum samples as early as 1980 as well as in the present study (20). In this

region, without recognized cases of HFRS, we found a 2.7% prevalence of subclinical Hantavirus infection in the general population similar to those geographic areas where HFRS is endemic (6). By indirect IFAT, all 20 positive individuals reacted against Hantaan virus and 16 against Seoul virus in similar titers. PRNT performed in 2 sera among positive general population showed no significant neutralizing antibodies against Hantaan and Seoul viruses as shown in Table 16, implying that Hantavirus infection in the surveyed population might be due to a serologically different serotype. IF antibodies against hantaviruses are group specific but PRN antibodies are type specific (15). It is worthy to mention that 9 out of 10 seropositive individuals who were given questionnaires had close contact with urban rats and wild rodents, although none had apparent clinical illness. We also demonstrated a high prevalence of Hantavirus infection (13.9%) among laboratory personnel working in close contact with rodents. In this case PRN tests showed higher antibody titers against Seoul than Hantaan virus. Four out of the 10 seropositive laboratory workers showed significant titers of IgM antibodies by ELISA test, indicating recent infection. ELISA antibodies against only Hantaan virus were measured since they cross react equally to Hantaan and Seoul virus.

Subclinical infection may possibly have been acquired by laboratory staff from infected laboratory rats since recent data showed that 15% of the laboratory rats assayed from the Microbiology Department, School of Medicine, University of Buenos Aires, and 70% of the experimental rats tested at the school of Medicine, Mendoza province, are infected with Hantavirus. Recently, HFRS patients in the tropic areas where this disease is not known to exist were diagnosed clinically as dengue, leptospirosis, scrub typhus, hepatitis, trombocytopenia and influenza (42).

Laboratory rats have been the source of Hantaan or Seoul virus infection in laboratory workers from Japan, Korea and Belgium. There were several outbreaks of HFRS among personnel taking care of laboratory animals in these countries (30,31,43, 44). Our findings showed that laboratory rats as well as some wild rodents in Argentina are infected with Hantavirus. In 3 out of 4 Institutions, 14 out of 62 rats were found to have IF antibodies against Hantaan virus. To date it is unknown how these laboratory rats became contaminated. Sera from positive laboratory rats were assayed for identification of serotype of virus by PRNT and it was proved that Seoul virus was causative agent. Unfortunately, sera from positive Calomys mice were not tested by PRNT because of limited amount of sera. Harbour rats yielded negative results perhaps due to the limited areas of collection and the very low seasonal population density.

Serological survey performed in wild rodents led to detection of Calomys musculinus infected with Hantavirus. This is the first naturally infected South American Cricetid reported so far. IF antibody titers were low but specificity was confirmed by repeated tests. Since this species is recognized as the main natural reservoir of Junin virus, the coexistance of these two viral infections, Hantavirus and Junin virus, should be throughly investigated. C. musculinus nests in corn fields and its populations are the most abundant in agroecosystems. The finding of natural Hantavirus infection in C. musculinus both in Buenos Aires of the Atlantic Coast and in Mendoza in the west, bordering the Andean Range, illustrates the considerable risk of human infection in this geographical area. The infection rate of Calomys mice and isolation of Hantavirus from the wild rodents in Argentina remains to be studied.

On the basis of our findings in this area, we can assume that there are two serotypes of Hantavirus; Hantaan-like virus in wild rodents and Seoul virus in laboratory rats. The world-wide distribution of subclinical human Hantavirus infection is again documented in Southern parts of Latin America and clinical cases of HFRS remains to be documented.

Preliminary results indicate that 4 distinct viruses compose the genus Hantavirus, Hantaan, Puumala, Seoul and Prospect Hill viruses (15). It appears that several subtypes of strains exist within each virus type by monoclonal antibodies and molecular biologic studies and epidemiological evidence suggests that other Hantaviruses still exist unrecognized in nature (49). A further serological classification is thus needed to clearly differentiate specific Hantaviruses and to aid in the recognition of new viruses. A new serotype of Hantavirus (Maaji virus) was isolated from Apodemus agrarius caught in west-coast of S. Korea and identified by PRNT and IFAT. Maaji virus is very close to Hantaan virus antigenically and it is almost impossible to differentiate the two virus by neutralizing antibodies but it can be readily differentiated by use of convalescent sera from Yugoslavian HFRS as shown in table 15. Anti-Hantaan serum has very high IF antibodies to Hantaan virus and very low antibodies to P.H. virus but anti-Maaji serum has high IF antibodies against both Hantaan and P.H. virus.

An animal model mimic to man for study of HFRS has long been sought by many scientists although Japanese investigators claimed they have successfully reproduced the disease in monkeys in 1944 (48). Now we have evidences that some new genetic strains of inbred hamsters could be found as an animal model since we found that 2 out of 4 inbred hamsters PD4 died 19-21 days after inoculation of Hantaan virus in our limited experiments. Inbred hamsters could be used as a broad spectrum animal for study of hantaviruses because not only the inbred hamsters produced good antibodies but supported multiplication of Hantaan,

Seoul and Puumala viruses in lungs and other organs after inoculation of the viruses intramuscularly.

In fact, a strain of Seoul virus was isolated from a Syrian hamster by accident when we bought Syrian hamsters from a local animal farm for study of leptospirosis in 1985. We found IF antibodies against Hantaan virus in sera from the hamsters when we bled blood from the hamsters prior to using them and then we demonstrated Hantavirus antigen in lungs from the seropositive hamster by IFAT. This result finally led us to investigate many strains of available hamsters for study of hantaviruses. It is too early to conclude that inbred hamsters PD4 are susceptible animal model mimic to man for HFRS because our experiments are limited and preliminary nature. Further well designed experiments to confirm the results and to develop an animal model in inbred hamsters are urgently needed.

CONCLUSION

1. We have confirmed serologically 701 HFRS among 2,311 suspect HFRS patients in Korea in 1987 and, 163 and 5 of them were ROK Army and US Army soldiers, respectively.
2. There were 209 cases of scrub typhus among 1,530 suspect HFRS patients and, 8 and 2 of them were ROK Army and US Army soldiers in Korea in 1987.
3. The peak of outbreak of HFRS was October-December but peak of scrub typhus was September-October in Korea.
4. The rate of infected _Apodemus agrarius_ with Hantaan virus in the US Marine and the ROK Army camps in Wuncheon was 16% and 9%, and the infection rate of _Apodemus agrarius_ with leptospira in the US Marine and the ROK camps was 19% and 11%, respectively. However, infection rate of _Apodemus_ mice with _R. tsutsugamushi_ in both Army camps was about same 32%.
5. Four strains of Hantaan virus were isolated from _Apodemus agrarius_ caught in Jinhae, Kyungsangnamdo, southern coast of S. Korea where HFRS were not reported but outbreaks of scrub typhus were confirmed previously.
6. A new serotype of Hantavirus was identified, Maaji virus, isolated from _Apodemus agrarius_ caught in Maaji, Kyunggido, Korea in 1980. Maaji virus is closely related to Hantaan virus but it can be differentiated from Hantaan with sera from some KHF and HFRS in Yugoslavia and Prospect Hill antiserum by PRNT.
7. Hantavirus infection was demonstrated not only among laboratory personnel and general population in Argentina and Uruguay, but also in experimental and wild rodents in Argentina. This is the first evidence of presence of Hantavirus infection in wild _C. musculinus_ and laboratory rats in Argentina.
8. A strain of Seoul virus was isolated from a Syrian hamster bought from a local animal farm in Seoul.
9. Inbred hamsters were susceptible and support multiplication of Hantaan, Seoul and Puumala viruses after inoculation of the viruses. Two out of 4 inbred hamsters PD4 died about 20 days after inoculation of a strain of Hantaan virus. Some strains of inbred hamsters are animal model candidates mimic to man for study of HFRS.

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